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Wadeable Streams Assessment: A Collaborative Survey of the Nation's Streams EPA 841-B-06-002

Appendix A 2006 Wadeable Streams Assessment Data Analysis Approach

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Appendix A

2006 Wadeable Streams Assessment: Data Analysis Approach

Overview

This appendix provides additional information to supplement the results and discussion presented in the 2006 Wadeable Streams Assessment (WSA). It is intended to provide a more technical reference than the report itself on the conceptual basis and the methods and procedures used for the WSA. Although it is intended to provide a comprehensive summary of these procedures, it is not intended to present additional data analysis results or an in-depth report of the design, sampling, or analysis protocol. For additional details, citations are provided.

Objectives of the WSA Assessment

The objective of the WSA assessment is to characterize the ecological condition of wadeable streams and rivers throughout the conterminous United States. The WSA is an ecological assessment of streams based on chemical, physical, and biological data. It employs a statistically-valid probability design stratified to allow estimates of the condition of streams on a national and regional scale. The two key questions the WSA addresses are

- To what degree are the Nation's wadeable streams in good, fair, and poor condition?
- What is the relative importance of the different stressors evaluated in the WSA?

The WSA is a collaboration among the U.S. Environmental Protection Agency (EPA), states, tribal nations, U.S. Geological Survey (USGS), and other partners. It is intended as a document for the public and Congress. It is not a technical document, but rather a report geared towards a broad audience, some with little or scientific background. This Technical Addendum is a supplemental document used to support the results in the WSA report. It describes the process used to collect, evaluate, and analyze data for the WSA. It outlines steps taken to assess the biological condition of the nation's freshwater resources and identify the relative impact of stressors on this condition. Results from the analysis are included in this 2006 WSA Report; the data collected and methods described will continue to be studied and used for future analyses.

The WSA data analysis procedures described in this addendum were developed from the input and experience of the participating cooperators and technical experts. Two small workgroups were held in the fall of 2005 to consider approaches for data analysis. Findings from these workshops were presented to a larger group of cooperators at the Wadeable Streams National Meeting in January 2006. Here, state agencies, universities, non-profits, EPA, and other federal agencies participated in a number of small breakout sessions where they discussed topics such as analysis options, data presentation, and reference sites. Discussions from these meetings were used to define the steps taken for the data analysis presented in the final report.

Reference Condition

To assess current ecological condition, it is necessary to compare measurements today to an estimate of expected measurements in a less-disturbed situation. Setting reasonable expectations for each indicator was one of the greatest challenges for the WSA. Because of the difficulty in estimating historical conditions for many WSA indicators, the 2006 WSA used “least-disturbed condition” as the reference condition. Least-disturbed condition can be defined as the best available chemical, physical, and biological habitat conditions given the current state of the landscape. Reference criteria describe the sites whose condition is “the best of what’s left.” Data from reference sites were used to develop the ecoregionally specific reference conditions against which test results could be compared.

Sources of Reference Sites

The reference sites used in the WSA came from two major sources:

1. Sites sampled during the WSA using consistent sampling protocols and analytical methods that were screened to meet ecoregional specific physical and chemical criteria. These included both sites selected randomly from the probability sample and sites hand-picked to be reference by best professional judgment and sampled using WSA methods as part of the WSA. For example, in the Eastern United States, states submitted 10 of their best reference sites to be sampled as part of the WSA.
2. Sample data provided by other agencies, universities, or states from sites that were deemed to be suitable as reference sites by best professional judgment. Based on recommendations from a technical workgroup and preliminary comparability work, external sources of reference sites were incorporated into the analysis portion of the assessment. These sites were either sampled with the same methodology as the WSA or had field and lab protocols with enough similarities that the data analysis group felt the data were comparable.

Screening WSA Site Data for Reference Condition

To identify reference sites for purposes of the WSA, we used the chemical and physical data we collected at each site (e.g., nutrients, turbidity, acidity, riparian condition) to determine whether any given site is in least-disturbed condition for its ecoregion. In the WSA, nine physical and chemical parameters were used to screen for reference sites, total nitrogen, total phosphorus, chloride, sulfate, acid-neutralizing capacity, turbidity, rapid habitat assessment score, percent fine substrate, and riparian disturbance index. If a site exceeded the screening value for any one stressor, it was dropped from reference consideration. Given that expectations of least-disturbed condition vary across ecoregions, the criteria values for exclusion varied by ecoregion. The nine aggregate level III ecoregions developed for the WSA were used to regionalize reference conditions (Table A-1). All sites in the WSA (both probability and hand-picked) that passed all criteria were considered to be reference sites for the WSA.

Table A-1. Macroinvertebrate Reference Sites

Ecoregion	Data Source		Total
	External	WSA	
Northern Appalachians (NAP)	114	27	141
Southern Appalachians (SAP)	354	35	389
Coastal Plains (CPL)	98	15	113
Upper Midwest (UMW)	68	12	80
Temperate Plains (TPL)	124	38	162
Northern Plains (NPL)	10	18	28
Southern Plains (SPL)	56	21	77
Western Mountains (WMT)	335	129	464
Xeric (XER)	132	39	171
Total	1,291	334	1,625

Note that the WSA did not use data on landuse in the watersheds for this purpose—sites in agricultural areas (for example) may well be considered least disturbed, provided that their chemical and physical conditions are among the best for the region. Additionally, the WSA did not use data on the biological assemblages themselves because these are the primary components of the stream and river ecosystems being evaluated and to use them would constitute circular reasoning.

Data Supplied from External Sources

Ideally, WSA investigators would have used reference sites picked in a consistent manner and sampled with identical protocols in all analyses. However, macroinvertebrate assessments require a large number of reference sites; more were available by screening WSA sites as described in Chapter 2.1.1. Many other investigators have used reference sites in their analyses. The WSA project team compiled a set of macroinvertebrate reference site data from external sources focusing on regions of the country where reference site data were limited. The major sources of supplemental macroinvertebrate data were the following:

- State agency data
- USGS National Ambient Water Quality Assessment (NAWQA) data
- Utah State University STAR grant data
- Earlier EPA Environmental Monitoring and Assessment Program (EMAP) and Regional Environmental Monitoring and Assessment Program (REMAP) data.

To be included in the WSA analyses, these data had to meet the following standards of macroinvertebrate sampling and laboratory analysis:

- A multi-habitat sampling method
- A minimum 300 organism lab count
- A minimum of genus level identification of insects, including Chironomids.

Sites incorporated from the external sources had varying levels of similarity to the WSA. Reference sites from the EPA EMAP and REMAP studies were sampled using the same methodologies as the WSA. Utah State University received a STAR grant to identify and sample reference sites in the western states using the same methodologies as the WSA. Because both of these sources of reference sites were sampled using the same methodologies, they are considered highly comparable to the WSA. A comparability study done on USGS NAWQA sites and WSA methods in high-gradient streams showed the results of the two methods were comparable in these high-gradient stream areas. USGS NAWQA sites from low-gradient streams were not included because of differences in methods. Sites from state agencies had to meet the previously mentioned criteria to be incorporated into the assessment. These sites were considered comparable based on best professional judgment of the technical workgroups and feedback from the national WSA meeting. It was not possible to screen the data, for example, for physical or chemical criteria; as such comprehensive data were not available for all these sites. The resulting reference site database had macroinvertebrate data from 1,625 sites, 334 WSA sites, and 1,291 external source sites.

Benthic Macroinvertebrate Assemblage

The taxonomic composition and relative abundance of different taxa that compose the benthic macroinvertebrate assemblage present in a stream have been used extensively in North America, Europe, and Australia to assess how human activities affect ecological condition (Barbour et al., 1995, 1999; Karr and Chu 1999). Two principal types of ecological indicators to assess condition based on benthic macroinvertebrates are currently prevalent: multimetric index and predictive models of taxa richness. The purpose of these indicators is to present the complex data represented within an assemblage in a way that is understandable and informative to resource managers and the public. Both approaches were recommended for use in the WSA by cooperators and participants at the WSA national meeting. The following chapters provide a general overview of the approaches used to develop ecological indicators based on benthic macroinvertebrate assemblages, followed by details regarding data preparation and the process used for each approach to arrive at a final indicator.

Overview: Macroinvertebrate Index and O/E Predictive Model Approaches

Multimetric indicators have been used in the United States to assess condition based on fish and macroinvertebrate assemblage data (e.g., Karr and Chu, 1999; Barbour et al., 1999; Barbour et al., 1995). The multimetric approach involves summarizing various assemblage attributes (e.g., composition, tolerance to disturbance, trophic and habitat preferences) as individual “metrics” or measures of the biological community. Candidate metrics are then evaluated for various aspects of performance, and a subset of the best performing metrics are combined into an index, typically referred to as a Macroinvertebrate Index of Biotic Condition (Macroinvertebrate Index).

The predictive model approach was initially developed in Europe and Australia and is becoming more prevalent within the United States. The approach estimates the expected taxonomic composition of an assemblage in the absence of human stressors (Hawkins et al., 2000; Wright, 2000), using a set of least-disturbed sites and other variables related natural gradients (e.g., elevation, stream size, stream gradient, latitude, longitude). The resulting models are then used to estimate the expected taxa composition (expressed as taxa richness) at each

stream site sampled. The number of expected taxa actually observed at a site is compared to the total number of expected taxa as an Observed Expected ratio (O/E index). Departures from a ratio of 1.0 indicate that the taxonomic composition in a stream sample differs from that expected under least-disturbed conditions.

Data Preparation: Standardizing Counts

The number of individuals in a sample was standardized to a constant number to provide an adequate number of individuals that was the same for nearly all samples and that could be used for both multimetric index development and O/E predictive modeling index. A subsampling technique involving random sampling without replacement was used to extract a true “fixed count” of 300 individuals from the total number of individuals enumerated for a sample (target count = 500 individuals).

Samples that did not contain at least 300 individuals were reviewed and retained for further analysis when appropriate (i.e., if the sampling effort was determined to be sufficient) because low counts can indicate a response to one or more stressors. For samples from sites classified as least disturbed, those with at least 250 individuals were retained.

Operational Taxonomic Units

To provide a nationally consistent database for the macroinvertebrates, taxonomic listings were reviewed for discrepancies. In some cases it was necessary to combine taxa to a coarser level of common taxonomy. This new combination of taxa is called the “Operational Taxonomic Unit” or OUT and improves the level of confidence in an overall assessment.

Autecological Characteristics

Autecological characteristics refer to specific ecological requirements or preferences of a taxon for habitat preference, feeding behavior, general behavior, and tolerance to human disturbance. These characteristics are prerequisites for the Macroinvertebrate Index, which incorporates various ecological attributes into its framework. A number of state/regional organizations and research centers have developed autecological characteristics for benthic macroinvertebrates in their region. For the WSA, a consistent national list of characteristics that consolidated and reconciled any discrepancies among the regional lists was developed and calibrated for use in a Macroinvertebrate Index.

Members of the data analysis group pulled together autecological information from five existing sources: the EPA Rapid Bioassessment Protocols document, the NAWQA national and northwest lists, the Utah State University list, and the EMAP Mid-Atlantic Highlands (MAHA) and Mid-Atlantic Integrated Assessment (MAIA) list. These five were chosen because they were thought to be the most independent of each other and the most inclusive taxa. A single national-level list was developed based on the decision rules outlined below.

Tolerance Values

Tolerance value assignments followed the convention for macroinvertebrates, ranging between 0 (least tolerant or most sensitive) to 10 (most tolerant). For each taxon, tolerance values from all five sources were reviewed, and a final assignment was made according to the following rules:

- If values from different lists were all < 3 (sensitive), final value = mean;
- If values from different lists were all > 3 and < 7 (facultative), final value = mean;
- If values from different lists were all > 7 (tolerant), final value = mean;
- If values from different lists spanned sensitive, facultative, and tolerant categories, best professional judgement was used, along with alternative sources of information (if available) to assign a final tolerance value;
- Tolerance values of 0–3 were considered “sensitive”; values of 8–10 were considered “tolerant”; and values of 4–7 were considered “facultative.”

Functional Feeding Group and Habit Preferences

In most cases, there was a high agreement among the five data sources. When discrepancies in functional feeding group (FFG) or habit preference assignments among the five primary data sources were identified, a final assignment was made based on the most prevalent assignment. In cases where there was no prevalent assignment, the workgroup examined why disagreements existed, flagged the taxon, and used best professional judgment to make the final assignment.

Macroinvertebrate Index Development

Two alternative approaches to developing a Macroinvertebrate Index for the WSA were evaluated. The first alternative was to develop separate, yet coordinated, Macroinvertebrate Indexes for each of the nine assessment regions. This approach recognizes the potential need for metrics to be selected and scored separately by region, but uses a single evaluation and scoring process so that the individual regional indexes can be combined into a single assessment without introducing regional bias. Each regional Macroinvertebrate Index was composed of a core set of metrics that performed best in that region.

The second alternative was to develop a single, universal index for the entire WSA study area. The universal Macroinvertebrate Index consisted of a single set of core metrics that performed adequately across all regions, but addressed regional biases by scoring metrics separately by assessment region, and used different thresholds in each assessment region to identify least-disturbed versus most-disturbed condition. After evaluating the results from both approaches, the regionally specific Macroinvertebrate Indexes were better able to discriminate least-disturbed from most-disturbed sites; therefore, the regional indexes were used to assess ecological condition for the WSA.

Metric Evaluation and Selection

Candidate metrics were derived from the benthic invertebrate count data and the autecological characteristics of each taxon. In most cases, three variants of each candidate metric were calculated: one based on taxa richness, one based on the proportion of individuals, and one based on the proportion of taxa. All candidate metrics were assigned to one of the following six categories representing different aspects of biotic integrity (Barbour et al., 1999; Karr, 1993; Karr et al., 1986; Stoddard et al., 2005)

- **Richness:** The number of different kinds of taxa.

- **Diversity:** Evenness of the distribution of individuals across taxa.
- **Composition:** The relative abundance of different kinds of taxa.
- **Functional feeding groups:** The Primary method for acquiring food.
- **Habit:** The habitat preference or dominant behavior, i.e., do taxa cling to substrates, or burrow into substrates?
- **Tolerance:** Often expressed as a general tolerance to stressors.

A series of performance evaluations was conducted to identify the best metric from each metric category. The evaluations were applied sequentially and by assessment region. Candidate metrics that failed a test were eliminated from additional consideration and testing.

- **Range test:** Candidate metrics that have a small (or narrow) range, or where most of the values are identical, are not likely to provide information that helps differentiate among sites. Richness metrics were eliminated if their range was less than 4. Proportional metrics having a range ≤ 0.1 were retained, but were considered to be poor performers. Metrics having more than 75% of the values the same were also eliminated.
- **Signal to noise (S:N) test:** “Signal to noise” is the ratio of variance among sites and the variance within a site (based on repeated visits to the same site). A low S:N value indicates a metric that cannot distinguish among sites very well. S:N ratios were calculated for each assessment region. Generally, candidate metrics having S:N values ≤ 1 were eliminated.
- **Responsiveness:** Responsiveness to disturbance was evaluated using standard statistical technique, an F-test, to determine if the mean metric values for least-disturbed and most-disturbed sites were statistically equivalent or distinct. Candidate metrics with $F \leq 1$ were eliminated.

Candidate metrics that passed all of the above tests were sorted by F values. Selection of the final metrics for inclusion in a Macroinvertebrate Index was conducted separately for each assessment region. The metric with the highest F value was selected first. The metric having the next highest F value that was from a different metric category was then selected. This process was repeated until one metric from all 6 metric categories was selected. As a final test, the selected metrics were evaluated for redundancy.

- **Redundancy:** Only metrics that did not contain redundant information were included in the final indexes. Inclusion of redundant metrics adds little information to the Macroinvertebrate Index, and may bias the index. We evaluated redundancy by using only the set of least-disturbed sites to avoid eliminating metrics that are correlated only because of their relationship to stressors that co-vary. A pairwise correlation analysis was conducted. Metrics having a Pearson correlation coefficient (r) > 0.71 were considered to be redundant. This value of r corresponds to a coefficient of determination (r^2) value of 0.5.

For each metric pair that was redundant, the metric selected for inclusion first (i.e., with the higher F value) was retained. The redundant metric was replaced with the metric from the same metric category that had the highest F value and was non-redundant.

Using the approach described above, final metrics selected for the regional Macroinvertebrate Indexes are shown in Table A-2.

Table A-2. Metrics used by ecoregion and nationally for the Macroinvertebrate Index

Final metrics selected for the regional Macroinvertebrate Indices were:

Metric	NAP	SAP	CPL	UMW	TPL	NPL	SPL	WMT	XER
EPT % Taxa	X					X		X	
EPT % Individuals					X		X		
Non-Insect % Individuals			X						X
Ephemeroptera % Taxa		X							
Chironomid % Taxa				X					
Shannon Diversity		X	X	X	X	X	X		
% Individuals in Top 5 Taxa	X							X	X
Scraper Richness	X	X			X	X	X	X	X
Shredder Richness			X	X					
Burrower % Taxa		X		X		X	X		
Clinger % Taxa	X		X					X	X
Clinger Richness					X				
Ephemeroptera Richness					X	X			
EPT Richness	X	X	X	X			X	X	X
Intolerant Richness							X		
Tolerant % Taxa		X	X					X	X
Hillsenhoff Biotic Index									
PTV 0-5.9 Richness						X			
PTV 0-5.9% Taxa	X								
PTV 8-10% Taxa				X	X				

Metric Scoring and Macroinvertebrate Index Calculation

Before being combined into an Macroinvertebrate Index, each metric was scored to translate results to a single scale (a continuous scale ranging from 0 to 10). For each regional index, each of the six metrics was scored separately by assessment region using a scheme intended to maximize differences in final index scores (Blocksom, 2003). Scoring was based on the distribution of metric values of all sites sampled. For metrics having the highest values at least-disturbed sites, values less than the 5th percentile were scored as 0 (floor value), while those with values equal to or greater than the 95th percentile were scored as 10 (ceiling value). All metric values in between were assigned a score based on a linear interpolation between the ceiling and floor values. For metrics having the highest values at most-disturbed sites, values less than the 5th percentile were scored as 10, while values greater than or equal to the 95th percentile were scored as 0. The final Macroinvertebrate Index score was calculated by first

summing the six metric scores. This total was then scaled to range from 0 to 100 by multiplying it by 1.666.

The regional indexes were evaluated by calculating a S:N ratio and F value as described in Chapter 3.3.1.

Modeling of Macroinvertebrate Index Condition class thresholds for the WSA

Previous large-scale assessments have converted Macroinvertebrate Index scores into classes of assemblage condition by comparing those scores to the distribution of scores observed at least-disturbed reference sites. If a site's index score was less than the 5th percentile of the reference distribution, it was classified as most-disturbed condition; those scores between the 5th and 25th percentile were classified as intermediate disturbance; and scores greater than the 25th percentile were classified as least-disturbed condition. This approach assumes that the distribution of index scores at reference sites reflects an approximately equal, minimum level of human disturbance across those sites. But this assumption did not appear to be valid for some of the nine assessment regions, which was confirmed by state and regional biologists at meetings to review the draft results. When reviewing reference sites, the variation in the quality of references between the individual regions indicates that the thresholds drawn using these reference conditions set unequal bars across the nation. Regions with high-quality reference sites had more stringent thresholds than regions with disturbed reference sites.

For the WSA, the project team performed a principal components analysis (PCA) of nine habitat and water chemistry variables that had originally been used to select Macroinvertebrate Index reference sites. The first principal component (Factor 1) of this PCA represented a generalized gradient of human disturbance. Index scores were weakly, but significantly, related to this disturbance gradient in five of the nine aggregate regions (Figure A-1), contrary to the assumption of approximately equal disturbance levels. Thus, index reference distributions from these regions are biased downward because they include somewhat disturbed sites that have low index scores, unless we account for this in the process of setting thresholds.

The regression models in Figure A-1 were used to adjust the Macroinvertebrate Index reference distributions in the five regions (Southern Appalachians [SAP], Temperate Plains [TPL], Northern Plains [NPL], Southern Plains [SPL], Western Mountains [WMT]) to reflect only the better reference conditions within a region, as indicated by lower disturbance scores (PCA Factor 1 scores). Figure A-2 explains the adjustment method and illustrates the method for the Western Mountains region. Following distribution adjustments, the Least/Intermediate and Intermediate/Most disturbed class thresholds for each region were defined by the 5th and 25th percentiles of that region's adjusted index distribution, as illustrated in Figure A-2. Macroinvertebrate Index threshold values can be found in Table A-3.

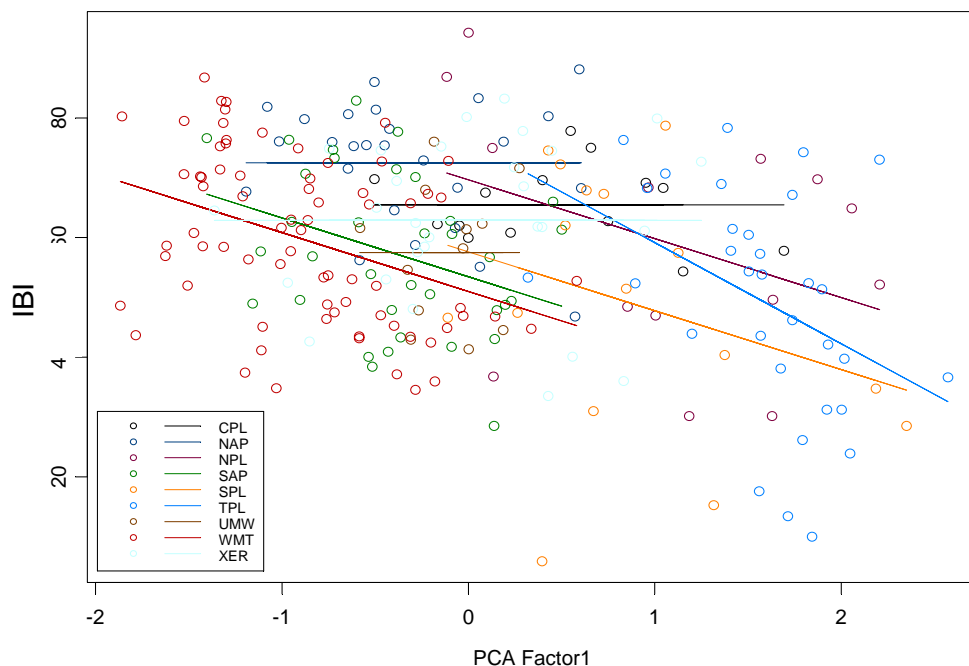


Figure A-1. Scatterplot and regression models of Macroinvertebrate Index versus PCA Factor 1 scores at reference sites, by region. Horizontal lines denote regions with no significant relationship.

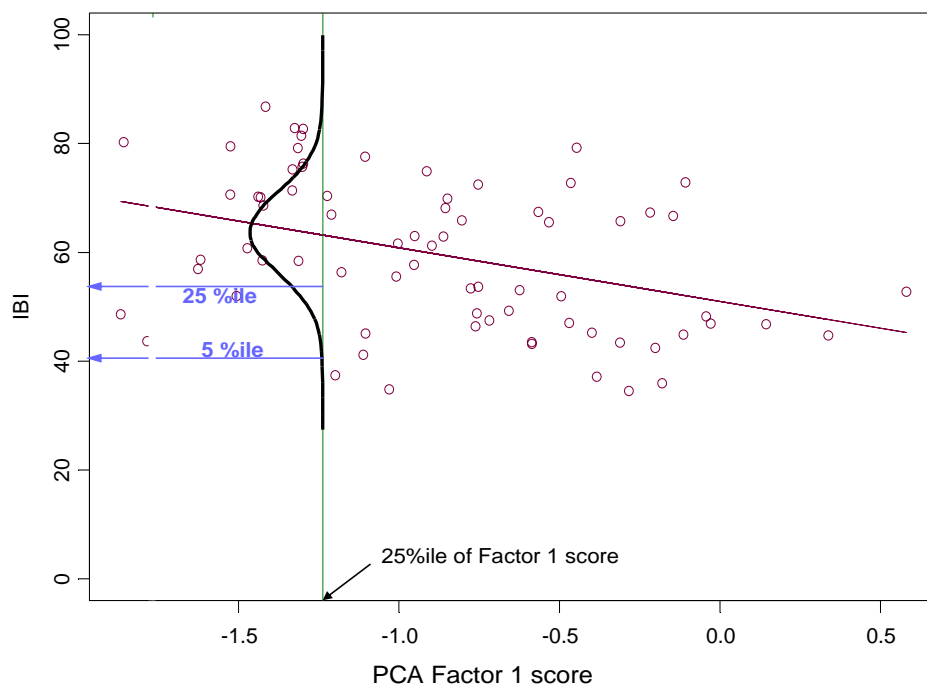


Figure A-2. Adjusting the Macroinvertebrate Index reference distribution and setting class thresholds for the Western Mountains (WMT) region. Points denote Macroinvertebrate Index and Factor 1 scores at all WMT reference sites; the line is a linear regression on those points. We assumed that index scores at a subset of the “better” reference sites would be normally distributed, with a mean value predicted by the regression from the 25th percentile of the PCA Factor 1 score.

The distribution's standard deviation is estimated by the pooled residual standard deviation obtained from regressions in all regions. Macroinvertebrate Index disturbance class thresholds (41 and 55) are given by the 5th and 25th percentile of the distribution at better reference sites.

Table A-3. Threshold values for the nine regional Macroinvertebrate Indexes.

Region	Least-Disturbed/ Intermediate	Intermediate/ Most-Disturbed
CPL	56	42
NAP	63	49
NPL	62	49
SAP	56	42
SPL	50	36
TPL	52	38
UMW	48	34
WMT	59	45
XER	53	40

O/E: Predictive (RIVPACS) Models

The second method used to assess ecological condition for the WSA was a predictive O/E model. The O/E model compares the observed benthic assemblage at a site to an expected assemblage derived from a population of reference sites. Stressors and anthropogenic impacts lead to a reduction in the number of taxa that are expected to be present under reference conditions. The predictive model approach is used by several states and is a primary assessment tool of Great Britain and Australia.

The O/E ratio predicted by the model for any site expresses the number of taxa found at that site (O), as a proportion of the number that would be expected (E) if the site was in least-disturbed condition. Ideally, a site in reference condition has an $O/E = 1.0$. An O/E value of 0.70 indicates that 70% of the expected taxa at a site were actually observed at the site. This is interpreted as a 30% loss of taxa relative to the site's predicted reference condition. However, O/E values vary among reference sites themselves, around the idealized value of 1.0, because such sites rarely conform to an idealized reference condition and because of model error and sampling variation. The standard deviation of O/E (Table A-4) indicates the breadth of O/E variation at reference sites. Thus, the O/E value of an individual site should not be interpreted as (1 – taxa loss) without taking account of this variability in O/E. Individual O/E values are most reliably interpreted relative to the entire O/E distribution for the reference sites.

A nationally-distributed collection of reference sites was first identified, drawn from a pool of sites whose macroinvertebrates were sampled using EMAP protocols. This pool included only WSA, EMAP-West, STAR-USU, USGS NAWQA, and MAHA/MAIA sites. Twenty percent of all reference sites were set aside to validate the models, and the remaining 80% were used to calibrate the models (Table A-4). Each site contributed a single sampled macroinvertebrate assemblage to model calibration and validation. Each sampled macroinvertebrate assemblage comprising more than 300 identified individuals was randomly subsampled to yield 300 individuals. These 300-count subsamples were used to build models and assess all WSA sites.

The predictive modeling approach assumes that expected assemblages vary across reference sites throughout a region due to natural (nonanthropogenic) environmental features such as geology, soil type, elevation, and precipitation. To model these effects, the approach first classifies reference sites based on similarities of their macroinvertebrate assemblages (Table A-4). A discriminant function model is then built to predict the membership of any site in these classes, using natural environmental features as predictor variables (Table A-4). The predicted occurrence probability of a reference taxon at a site is then predicted to be the weighted average of that taxon's occurrence frequencies in all reference site classes, using the site's predicted group membership probabilities in the classes as weights. Finally, E for any site is the sum, over a subset of reference taxa, of predicted taxon occurrence probabilities, whereas O is the number of taxa in that subset that were observed to be present at the site. The subset of reference taxa used for any site was defined as those taxa with predicted occurrence probabilities exceeding 0.5 at that site.

Final predictive models performed better than corresponding null models (no adjustment for natural-factor effects), as judged by their smaller standard deviation of O/E across calibration sites (Table A-4).

Similar to the Macroinvertebrate Index, two scaled approaches were used to develop the O/E model. A national model was initially developed to predict taxa loss at sites, and three models were developed for WSA usage, together covering the conterminous United States (Table A-4). The regional models performed better and were used in the WSA to predict taxa loss at the sites.

The three final regional models were applied to estimate O/E for 1354 WSA sites that were sampled for benthic macroinvertebrates, depending on each site's regional location. Predictions could not be made for 36 WSA sites because the predictor data was either missing or outside the model's experience.

Table A-4. WSA predictive models.

Model Name	Eastern Highlands	Plains and Lowlands	West
Regions covered	NAP, SAP	CPL, UMW, TPL, NPL, SPL	WMT, XER
Number of calibration sites	193	138	519
Number of validation sites	43	40	123
Number of site classes	11	11	31
Discriminant function predictor variables	Site longitude, mean of minimum annual temperature, mean number of wet days per year, watershed area, Julian day of sampling	Julian day of sampling, elevation, mean number of frost-free days per year, mean annual precipitation, watershed area, stream gradient	Site longitude, Julian day of sampling, watershed area, mean annual precipitation, mean of minimum annual temperature, elevation, stream gradient
Standard deviation of O/E at calibration sites:			
Predictive model	0.16	0.27	0.19
Null model	0.21	0.29	0.26

Physical Habitat Condition Assessment

An assessment of stream physical habitat condition was a major component of the WSA. Of many possible general and specific stream habitat indicators measured in the WSA (see Kaufmann et al., 1999), the WSA chose streambed excess fine sediments, habitat cover complexity, riparian vegetation, and riparian human disturbances in this assessment. These four indicators are generally important throughout the United States. Furthermore, the project team had reasonable confidence in factoring out natural variability to determine expected values and the degree of anthropogenic alteration of the habitat attributes represented by these indicators.

Streambed Sediments

Streambed characteristics (e.g., bedrock, cobbles, silt) are often cited as major controls on the species composition of macroinvertebrate, periphyton, and fish assemblages in streams (Hynes, 1972; Cummins, 1974; Platts et al., 1983; Barbour et al., 1997). Along with bedform (e.g., riffles and pools), streambed particle size influences the hydraulic roughness and, consequently, the range of water velocities in a stream channel. It also influences the size range of interstices that provide living space and cover for macroinvertebrates and smaller vertebrates. Accumulations of fine substrate particles (excess fine sediments) fill the interstices of coarser bed materials, reducing habitat space and its availability for benthic fish and macroinvertebrates (Platts et al., 1983; Hawkins et al., 1983; Rinne, 1988). In addition, these fine particles impede circulation of oxygenated water into hyporheic habitats. Streambed characteristics are often sensitive indicators of the effects of human activities on streams (MacDonald et al., 1991; Barbour et al., 1997). Decreases in the mean particle size and increases in streambed fine sediments can destabilize stream channels (Wilcock, 1997; Wilcock, 1998) and may indicate increases in the rates of upland erosion and sediment supply (Lisle, 1982; Dietrich et al., 1989).

Unscaled measures of surficial streambed particle size, such as percent fines or D_{50} , can be useful descriptors of streambed conditions. In a given stream, increases in percent fines or decreases in D_{50} may result from anthropogenic increases in bank and hillslope erosion. However, a great deal of the variation in bed particle size we see among streams is natural—the result of differences in stream or river size, slope, and basin lithology. The power of streams to transport progressively larger sediment particles increases in direct proportion to the product of flow depth and slope. Steep streams tend to have coarser beds than similar sized streams on gentle slopes. Similarly, the larger of two streams flowing at the same slope will tend to have coarser bed material because the deeper flow has more power to scour and transport fine particles downstream (Leopold et al., 1964; Morisawa, 1968). For these reasons, we “scale” bed particle size metrics, expressing bed particle size in each stream as a deviation from that expected as a result of its size, power, and landscape setting. Relative Bed Stability (RBS) is a scaled-bed particle size metric and is the metric that is used to determine the streambed sediment indicator for the WSA.

Although many human activities directly or indirectly alter the size of streambed material, bed particle sizes also vary naturally in streams with different drainage areas, slopes, and surficial geologies (Leopold et al., 1964; Morisawa, 1968). The particle size composition of a streambed depends on the rates of supply of various sediment sizes to the stream and the rates at which the flow takes them downstream (Mackin, 1948). Topography, precipitation, and land cover influence sediment supply to streams, but the source of sediments is the basin soil and

geology, and supplies are greater where these materials are inherently more erodible. Once sediments reach a channel and become part of the streambed, their transport is largely a function of channel slope and discharge during floods (in turn, discharge is largely dependent upon drainage area, precipitation, and runoff rates). However, a stream or river's competence and capacity to transport sediments can be greatly altered by the presence of such features as large woody debris and complexities in channel shape (e.g., sinuosity, pools, changes in width/depth ratio). The combination of these factors determines the depth and velocity of streamflow and the shear stress (erosive force) that it exerts on the streambed. The streambed sediments indicator used in the WSA to evaluate bed stability and streambed excess fine sediments compares the actual particle sizes observed in a streambed with a calculation of the sizes of particles that can be mobilized by that stream. Values of streambed sediments lower than reference expectations generally indicate excess fine sediments from soil erosion, although unstable streambeds can also result from hydrologic alteration that increases the size or frequency of floods. Values of streambed sediments higher than reference expectations can indicate anthropogenic coarsening or armoring of streambeds, but streams containing substantial amounts of bedrock may also have very high streambed sediments score. At this time, it is difficult to determine the role of human alteration in stream coarsening on a national scale. For this reason, we currently report only on the "low end" of streambed sediments relative to reference conditions, generally indicating streambed sediments associated with human disturbance of stream drainages and riparian zones.

Many researchers have scaled observed stream reach or riffle particle size (e.g., median diameter D_{50} , or geometric mean diameter D_{gm}) by the calculated mobile, or "critical" bed particle diameter (D_{cbf}), in the stream channel. The scaled median streambed particle size is expressed as Relative Bed Stability (RBS), calculated as the ratio D_{50}/D_{cbf} (Dingman, 1984; Gordon et al., 1992), where D_{50} is based on systematic streambed particle sampling ("pebble counts") and D_{cbf} is based on the estimated streambed shear stress at bankfull flows. Kaufmann et al. (1999) modified the calculation of D_{cbf} to incorporate large wood and pools, which can greatly reduce shear stress in complex natural streams. They also formulated the calculation of both D_{gm} and D_{cbf} so that RBS could be estimated from physical habitat data obtained from large-scale regional ecological surveys such as WSA. RBS is quantified as the ratio of observed bed surface particle diameter divided by the "critical" or mobile particle diameter calculated for a given streamflow condition (Dingman, 1984). It is the inverse of the streambed "fining" measure calculated by Buffington and Montgomery (1999a; 1999b), and is conceptually similar to the "Riffle Stability Index" of Kappesser (2002) and the bed stability ratio discussed by Dietrich et al. (1989).

When evaluating the stability of whole streambeds (vs. individual bed particles), observed substrate is typically represented by the median surface particle diameter (e.g., D_{50}) or the geometric mean diameter (D_{gm}). To characterize the actual substrate particle size distribution in a stream channel, WSA field protocols followed the widely accepted procedure (e.g., Platts et al., 1983; Bauer and Burton, 1993) of employing a systematic "pebble count," as described by Wolman (1954). Observed bed particle size was calculated as the geometric mean particle diameter from systematic "pebble counts" of 105 particles along the stream bed.

To calculate critical (mobile) bed particle diameter in a natural stream, it is necessary to estimate average streambed tractive force, or shear stress, for establishing a common reference flow condition likely to mobilize the streambed. Bankfull discharge is typically chosen for this purpose because the shear stress under these conditions can be estimated from field evidence

observed during low flow in most regions. Bankfull flows are large enough to erode the stream bottom and banks, but frequent enough (return interval of one to two years) not to allow substantial growth of upland terrestrial vegetation (Harrelson et al., 1994; Kaufmann et al., 1999). Consequently, in many regions, it is these flows that have determined the width and depth of the channel, so the depth of one- to two-year floods can be approximated from the depth of the bankfull channel when evaluated in the field at low flow (Dunne and Leopold, 1978; Leopold, 1994). The WSA approach for estimating the critical diameter for bed particles in a stream is based on sediment transport theory (Simons and Senturk, 1977). This establishes an estimate of the average streambed shear stress or erosive tractive force on the bed during bankfull flow, based on quantitative estimates of bankfull flow depth, slope, channel shape, and roughness. Stream channels can be very complex, exhibiting a wide range in local bed shear stress due to small-scale spatial variation in slope, depth, and roughness within a channel reach (Lisle et al., 2000). The influence of large-scale channel roughness can be very important in determining bed stability, so we modified Dingman's (1984) RBS formulation to accommodate losses in shear stress resulting from large woody debris and channel complexity (Kaufmann et al., 1999; Kaufmann et al., in preparation). These roughness elements reduce shear stress and, therefore, critical diameter in streams flowing at a given depth and slope. Compared with simple or hydraulically "smooth" channels, shear stress is reduced in streams with large roughness elements, thereby increasing the stability of fine particles.

Finally, we calculated RBS as the reach-wide geometric mean substrate diameter divided by the bankfull critical diameter ($RBS = D_{gm} / D_{cbf}$), typically expressing it as the WSA variable LRBS_bw5, which is $\text{Log}_{10}(RBS)$. Similarly, $\text{Log}_{10}(RBS) = \text{Log}_{10}(D_{gm}) - \text{Log}_{10}(D_{cbf})$. The equivalent formula, expressed in WSA variables is $LRBS_bw5 = LSUB_dmm - LDMB_bw5$.

In interpreting RBS on a regional scale, Kaufmann et al. (1999) argued that, over time, streams and rivers adjust sediment transport to match supply from natural weathering and delivery mechanisms driven by the natural disturbance regime. This indicates that RBS in appropriately stratified regional reference sites should be evaluated in a range characteristic of the climate, lithology, and natural disturbance regime.

Values of the RBS Index that are either substantially lower (finer, more unstable streambeds) or higher (coarser, more stable streambeds) than those expected based on the range found in least-disturbed reference sites within an ecoregion are considered to be indicators of ecological stress. Excess fine sediments can destabilize streambeds when the supply of sediments from the landscape exceeds the ability of the stream to move them downstream. This imbalance results from numerous human uses of the landscape, including agriculture, road building, construction, and grazing. Lower than expected streambed stability may result either from high inputs of fine sediments (erosion) or increases in flood magnitude or frequency (hydrologic alteration). When low RBS results from fine sediment inputs, stressful ecological conditions result from fine sediments filling in the habitat spaces between stream cobbles and boulders.

In-stream Fish Habitat

The most diverse fish and macroinvertebrate assemblages are found in streams and rivers that have complex forms of habitat, including large wood, boulders, undercut banks, and tree roots. When other needs are met, complex habitat with abundant cover should generally support greater biodiversity than simple habitats that lack cover (Gorman and Karr, 1978; Benson and

Magnuson, 1992). Human use of streams and riparian areas often results in the simplification of this habitat, with potential effects on biotic integrity.

In-stream fish habitat is difficult to quantify. For this assessment, we use a measure (XFC_NAT in Kaufmann et al., 1999) that sums the amount of in-stream habitat consisting of undercut banks, boulders, large pieces of wood, brush, and cover from overhanging vegetation within a meter of the water surface, all of which are estimated visually by WSA field crews. The WSA Physical Habitat protocols provide estimates for nearly all of the following components of complexity identified during EPA's 1992 stream monitoring workshop (Kaufmann, 1993):

- Habitat Type and Distribution (e.g., Bisson et al., 1982; O'Neill and Abrahams, 1984; Frissell et al., 1986; Hankin and Reeves, 1988; Hawkins et al., 1993; Montgomery and Buffington, 1993, 1997, 1998).
- Large Woody Debris count and size (e.g., Harmon et al., 1986; Robison and Beschta, 1990).
- In-Channel Cover: Percentage areal cover of fish concealment features, including undercut banks, overhanging vegetation, large woody debris, and boulders (Hankin and Reeves, 1988; Kaufmann and Whittier, 1997)
- Residual pools, channel complexity, and hydraulic roughness (e.g., Lisle, 1992; Lisle, 1987; Kaufmann, 1987a; Kaufman, 1987b; Robison and Kaufmann, 1994)
- Width and depth variance and bank sinuosity (Kaufmann 1987a; Moore and Gregory, 1988; Madej, 2001;).

In-stream fish habitat and the abundance of particular types of habitat features differ naturally with stream size, slope, lithology, flow regime, and potential natural vegetation. For example, boulder cover will not occur naturally in streams draining deep deposits of loess or alluvium that do not contain large rocks. Similarly, large wood will not be found naturally in streams located in regions where riparian or upland trees do not grow naturally. Though the combined cover index XFC_NAT partially overcomes these differences, we set stream-specific expectations for habitat complexity metrics based on region-specific reference sites.

Riparian Vegetative Cover

The importance of riparian vegetation to channel structure, cover, shading, nutrient inputs, large woody debris, wildlife corridors, and as a buffer against anthropogenic disturbance is well recognized (Naiman et al., 1988; Gregory et al., 1991). Riparian vegetative cover not only moderates stream temperatures through shading, but also increases bank stability and the potential for inputs of coarse and fine particulate organic material. Organic inputs from riparian vegetation become food for stream organisms and provide structure that creates and maintains complex channel habitat.

The presence of a complex, multi-layered vegetation corridor along streams and rivers is a measure of how well the stream network is buffered against sources of stress in the watershed. Intact riparian areas can help reduce nutrient and sediment runoff from the surrounding landscape, prevent bank erosion, provide shade to reduce water temperature, and provide leaf litter and large wood that serve as food and habitat for stream organisms. The presence of canopy trees in the riparian corridor indicates longevity; the presence of smaller woody vegetation

typically indicates that riparian vegetation is reproducing and suggests the potential for future sustainability of the riparian corridor.

For the WSA, we evaluated the cover and complexity of riparian vegetation based the metric XCMGW, which is calculated from visual estimates of the areal cover and type of vegetation in three layers (the ground layer, woody shrubs, and canopy trees) made by WSA field crews. XCMGW is a combined measure of the cover of woody vegetation summed over the three vegetation layers, giving an indication of the abundance of vegetation cover and its structural complexity. Its theoretical maximum is 3.0 if there is 100% cover in each of the three vegetation layers. The separate measures of large and small diameter trees, woody and non-woody mid-layer vegetation, and woody and non-woody ground cover were all visual estimates of areal cover. XCMGW gives an indication of the longevity and sustainability of perennial vegetation in the riparian corridor (Kaufmann et al, 1999).

Riparian Disturbance

Agriculture, buildings, and other evidence of human activities in the stream channel and its riparian zone may, in themselves, serve as indicators of habitat quality. They may also serve as diagnostic indicators of anthropogenic stress. EPA's 1992 stream monitoring workshop recommended field assessment of the frequency and extent of both in-channel and near-channel human activities and disturbances (Kaufmann, 1993). In-channel disturbances include channel revetment, pipes, straightening, bridges, culverts, and trash. Near-channel riparian disturbances include buildings, lawns, roads, pastures, orchards, and row crops. The vulnerability of the stream network to potentially detrimental human activities increases with the proximity of those activities to the streams themselves. For this assessment, we use a direct measure of riparian human disturbance that tallies eleven specific forms of human activities and disturbances (e.g., roads, landfills, pipes, buildings, mining, channel revetment, cattle, row crop agriculture, silviculture) at 22 separate locations along the stream reach, and weights them according to how close to the channel they are observed (W1_HALL in Kaufmann et al., 1999). The index generally varies from 0 (no observed disturbance) to 6 (e.g., four types of disturbance observed in the stream, throughout the reach; or six types observed on the banks, throughout the reach). Although direct human activities certainly affect riparian vegetation complexity and layering measured by the Riparian Vegetation Index, the Riparian Disturbance Index is more encompassing and differs by being a direct measure of observable human activities that are presently or potentially detrimental to streams.

Setting Expected and Altered Values for Physical Habitat Indicators

Like most chemical and biological indicators, those for physical habitat commonly vary according to their geomorphic and ecoregional setting. We defined ecoregionally specific reference conditions for Streambed Sediments, In-stream fish habitat (XFC_NAT), and Riparian Vegetative Cover (XCMGW) based on percentiles of the statistical distributions of values of these variables measured in reference sites within each ecoregion. Reference sites were screened using a set of chemistry and stressor/habitat variables that did not include the variable of interest (e.g., no sediment variables were used in screening reference sites for streambed sediments). Within any given ecoregion, streambed particle size varies considerably, so the formulation of the streambed sediment variable was used as an indicator to factor out most of the expected

variability in streambed particle size associated with differences in the size and gradient of streams within each ecoregion.

Table A-5 shows the percentiles used to determine habitat indicator threshold values in the aggregated ecoregions named (e.g., 5th/25th means that we used the 5th percentile of reference sites to designate the threshold between intermediate and most-disturbed and the 25th percentile of the reference sites to designate the thresholds between intermediate and least-disturbed sites.)

Table A-5. Habitat Indicator Threshold Values

Streambed Sediments:	
10th/ 25th	CPL, NAP, NPL, SAP, SPL, TPL, XER
5th/ 25th	All other Ecoregions
In-stream Fish Habitat:	
25th/ 50 th	CPL, NPL, SPL, TPL
10th/ 35th	XER
5th/ 25th	All other Ecoregions
Riparian Vegetative Cover:	
25th/ 50 th	CPL, NPL, SPL, TPL
5th/ 25th	All other Ecoregions

Note that percentiles for Streambed Sediments and In-stream Fish Habitat were done separately for each of four subregions within the aggregated WMT ecoregion.

Riparian Disturbance Threshold

We did not set thresholds of alteration for this indicator based on the reference distribution. W1_HALL, the database variable name for this indicator, is a direct measure of human disturbance “pressure” – unlike the other habitat indicators, which are actually measures of habitat response to human disturbance pressures. It is very difficult to define what relatively undisturbed riparian areas are without using a screen based on these human disturbance tallies (i.e., W1_HALL). For this reason, we took a different approach for setting riparian disturbance thresholds, defining least-disturbed sites as those with W1_Hall < 0.33 and most-disturbed sites as those with W1_HALL > 1.5 in all ecoregions. A value of 1.5 means that at 22 locations in the stream, the field crews found 1 of 11 types of human disturbance within the stream or right on its banks. A value of 0.33 means that one type of human disturbance was observed at one-third of the 22 riparian plots along a sample stream.

Water Chemistry Analysis

Four chemical stressors are summarized in the WSA report: total nitrogen, total phosphorus, acidity and salinity. For acidity, threshold values were determined based on values derived during the NAPAP program. Sites with acid neutralizing capacity (ANC) less than zero were considered acidic. Those with dissolved organic carbon (DOC) greater than 10 mg/L were classified as organically acidic (natural). Acidic sites with DOC less than 10 and sulfate less than 300 µeq/L were classified as acidic deposition impacted, those with sulfate above 300 were acid

mine drainage impacted. Sites with ANC between 0 and 25 $\mu\text{eq/L}$ were considered acidic deposition influenced, but not currently acidic.

Salinity and nutrient classes were divided into low, medium, or high classes. Salinity classes were defined by specific conductance using ecoregional specific values (Table A-6). Total nitrogen and phosphorus were classified using a method similar to that used for Macroinvertebrate Index classes using deviation from reference by aggregate ecoregion. For nutrients, the value at the 25th percentile of the reference distribution was selected for each region to define the least-disturbed condition class (low-medium boundary). The 5th percentile of the reference distribution defines the most-disturbed condition class (Table A-6). For setting nutrient class boundaries, only reference sites from the screened WSA dataset were used. Because nutrients were the focus, the two nutrient screening levels used in defining reference sites were dropped and the other seven screening factors were used by themselves to identify a set of “nutrient reference sites.” Before calculating percentiles from this set of sites, outliers (values outside 1.5 times the interquartile range) were removed.

Table A-6. Nutrient and Salinity Category Criteria for WSA Assessment

Ecoregion	Salinity as Conductivity ($\mu\text{S/cm}$) Low-Medium	Salinity as Conductivity ($\mu\text{S/cm}$) Medium-High	Total N ($\mu\text{g/L}$) Low-Medium	Total N ($\mu\text{g/L}$) Medium-High	Total P ($\mu\text{g/L}$) Low-Medium	Total P ($\mu\text{g/L}$) Medium-High
CPL	500	1000	1092	2078	56.3	108
NAP	500	1000	329	441	8.2	15.7
SAP	500	1000	296	535	17.8	24.4
UMW	500	1000	716	1300	21.6	44.7
TPL	1000	2000	1750	3210	165	338
NPL	1000	2000	948	1570	91.8	183
SPL	1000	2000	698	1570	52.0	95.0
WMT	500	1000	131	229	14.0	36.0
XER	500	1000	246	462	35.5	70.0

Quality Assurance Summary

The WSA has been designed as a statistically valid report on the condition of wadeable streams at multiple scales, i.e., ecoregion (Level II), EPA region, and national, employing a randomized site selection process. The WSA is meant to complement the efforts of the EMAP Ecological Assessment of Western Streams and Rivers (EMAP West); therefore, it uses the same EMAP-documented and tested field methods for site assessment and sample collection as used by EMAP West. The WSA collected data on macroinvertebrates, water chemistry and physical habitat.

Key elements of the Quality Assurance (QA) program include:

- **Quality Assurance Project Plan** – A Quality Assurance Project Plan (QAPP) was developed and approved by a QA team consisting of staff from EPA’s Office and Wetlands Oceans and Watersheds (OWOW) and Office of Environmental Information (OEI) and a Project QA Officer. All participants in the program signed

- an agreement to follow the QAPP standards. Compliance with the QAPP was assessed through standardized field training, site visits, and audits. The QAPP addresses all levels of the program, from collection of field data and samples and the laboratory processing of samples to standardized/centralized data management.
- **Field training and sample collection** – EPA provided 9 training sessions throughout the study area (with at least one EMAP instructor in each session) for 162 field crew members of 33 field teams. All field teams were audited on site within the first few weeks of fieldwork. Adjustments and corrections were made on the spot for any field team problems. To assure consistency, EPA supplied standard sample/data collection equipment and site container packages. 748 random site, reference site, and repeat site samples were collected.
 - **Water chemistry laboratory QA procedures** – WSA used the same single lab as did EMAP West for all water chemistry samples. The Western Ecology Division (WED) was responsible for QA oversight in implementing the WSA QAPP and lab standard operating procedures (SOPs) for sample processing.
 - **Benthic laboratory QA procedures** – WSA used nine benthic labs, all nine were audited for adherence to the WSA QAPP/SOP for benthic sample processing. This included internal quality control (QC) checks on sorting and identification of benthic organisms and the use of the Integrated Taxonomic Information System for correctly naming species collected, as well as the use of a standardized data management system. Independent entomologists were contracted to perform QC analysis of 10% of each lab's samples (audit samples).
 - **Benthic sample QC findings** – Two of the nine benthic labs satisfied the QAPP measurement objectives, while the remaining seven labs were required to implement corrective actions and are subject to a second round of QC checks. The corrective actions were due to database entry errors, incomplete QC samples, or differences in number of taxonomic groups identified to target meeting or beyond. The second round of benthic QC resulted in all but one lab meeting the measurement objectives.
 - **Entry of field data** – WSA used the EMAP West data management structure, i.e., the same standard field forms for data collected in the field, with centralized data entry through scanning in to electronic data files. Internal error checks were used to confirm data sheets were filled out properly.
 - **Records management** – These records include (1) planning documents, such as the QAPP, SOPs, and assistance agreements and (2) field and laboratory documents, such as data sheets, lab notebooks, and audit records. These documents are ultimately to be maintained at EPA. All data are archived in the STORET data warehouse at www.epa.gov/STORET.

For more information on the Quality Assurance procedures, refer to the EPA Web site at www.epa.gov/owow/streams/survey/streams/survey.

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